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Novel antigens used to detect cell-mediated immune responses over time in *Mycobacterium avium* subsp. *paratuberculosis* infected cattle

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Background

Paratuberculosis is a chronic, granulomatous enteric infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in ruminants. Early-stage MAP infection can be detected using diagnostics for cell mediated immune responses, e.g. the whole blood interferon gamma (IFN-γ) test. Available IFN-γ tests are using purified protein derivatives of MAP (PPDj) which are crude products consisting of undefined antigens with possible cross reactions toward other environmental bacteria.

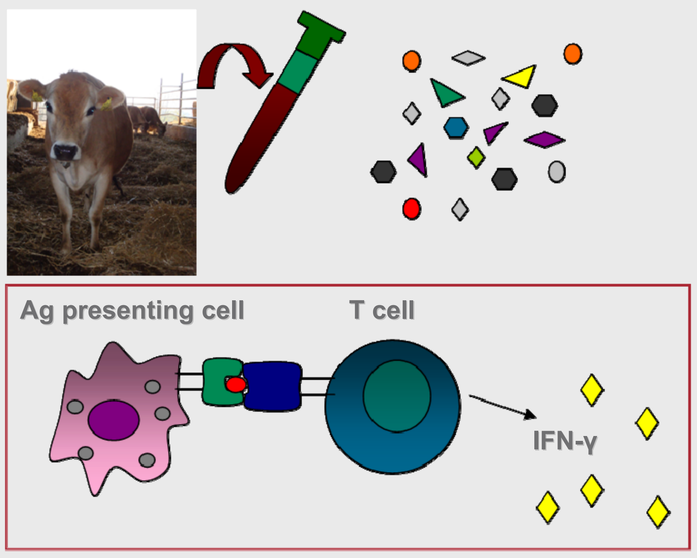
Objective

The objective was to optimize the IFN-γ test using different types of novel antigens for stimulation to determine if some antigens could be excluded or combined.

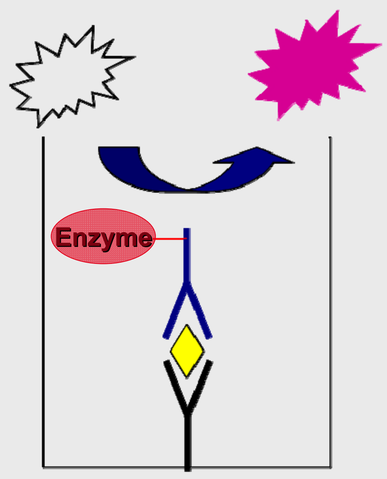
Methods

Fourteen novel antigens were selected for testing.
Blood samples were collected 3 times with 4 and 5 week intervals from the same 30 heifers 15-24 months of age in a herd with known MAP infection.
IFN-γ test protocol:

1) Stimulation of whole blood with MAP antigens



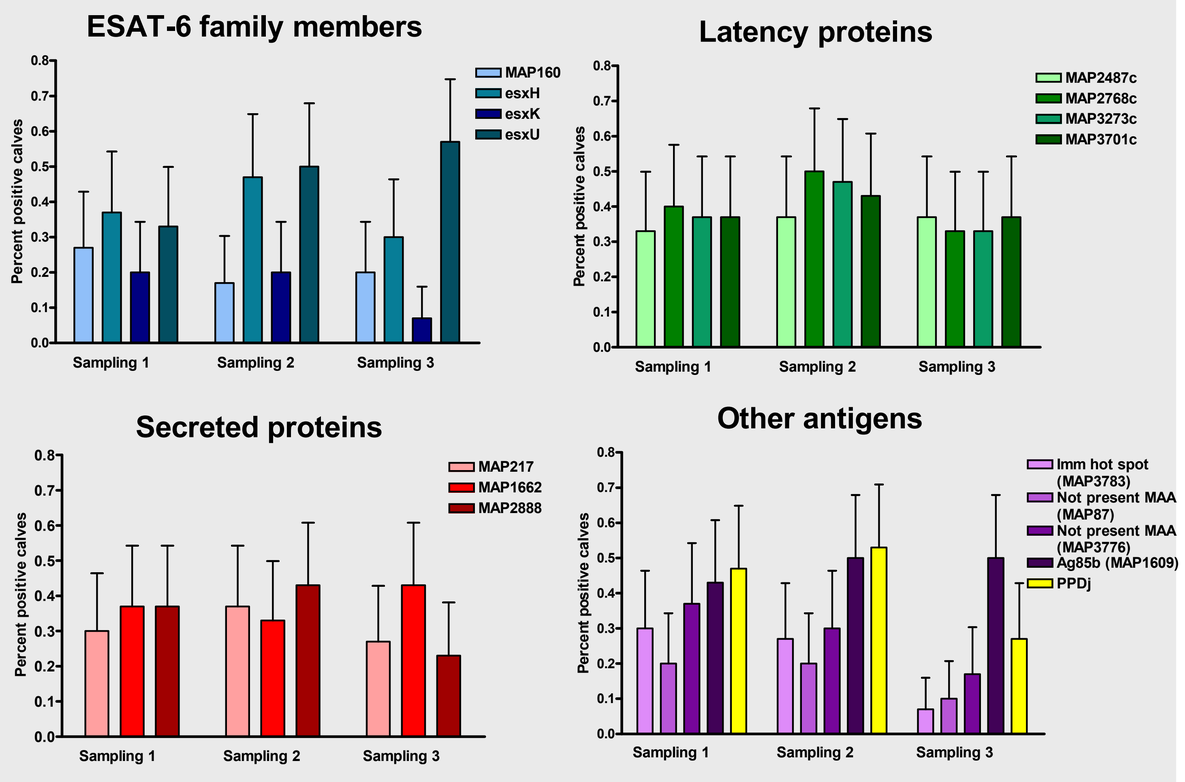
2) IFN- γ detection by ELISA



Conclusions

- PPDj stimulations tested approximately 50% of the animals as test positive at the two first samplings, and less than 30% on the third sampling.
- Ag85b tested 50% of the animals positive at all three samplings.
- The groups of latency proteins and secreted proteins tested approximately 40% of the animals test positive. Latency proteins showed the most consistent test results.
- ESAT-6 family proteins gave fluctuating test responses.

Results



IFN-γ responses = IFN-γ produced in response to specific antigen - IFN-γ produced in response to PBS-stimulation.

Interpretation

Cut-offs used to discriminate test-positives and test-negatives for each antigen was based on samples from 60 heifers 15-24 months of age from a non-infected herd.